A008

PRESENCE OF TISSUE FACTOR AND OTHER COMPONENTS OF ATHEROSCLEROSIS IN HUMAN AORTIC VALVE STENOSIS

J. BREYNE [1], F. JUTHIER [1,2], S. MARECHAUX [1,2], C. ZAWADZKI [1,2], D. CORSEAUX [1,3], A. VINCENTELLI [1,2], T. LE TOURNEAU [1,2], B. JUDE [1,2]
[1] *EA-2693, University of Lille II, Lille, France*
[2] *Cardiac Surgery Department, Echocardiography and Physiology Laboratories and Haematology Department, CHRU, Lille, France*
[3] *University of Lille I, Lille, France*

Background — It is now generally accepted that calcific aortic valve disease is an atherosclerotic-like process. Recent studies in an experimental model of aortic valve sclerosis demonstrated the presence of tissue factor (TF), the main contributor to atherosclerotic plaque thrombogenicity, in diseased valve leaflets. We assessed the hypothesis that human aortic valve disease is an atherosclerotic-like process in which TF plays an important role and evaluated the valvular expression and localization of TF and other components of atherosclerosis.

Methods — Calcified aortic valves (n=52) were obtained from patients undergoing aortic valve replacement. Leaflet structure, cellular and lipid infiltration and expression of TF, its inhibitors, VEGF and other components of atherosclerosis were evaluated by histological and immunohistochemical staining. TF, TFPI, osteopontin, MMP-9, TIMP-1 and VEGF antigen were measured by ELISA and TF and alkaline phosphatase activity were determined using chromogenic assays. Finally, we performed semi-quantification of TF transcripts by RT- PCR and further analyzed protein expression by Western blot.

Results — Histological and immunohistochemical staining of the valve leaflets revealed neovascularisation at the centre of the lesions, overall macrophage and myofibroblast infiltration and the abundant presence of MMP-9. On the other hand, TF and TFPI were associated with calcification and extracellular lipid deposits in the fibrosa and the subendothelial layer of the aortic side of the leaflets. Correspondingly, TF antigen and activity were found to be higher in calcified regions of the valve leaflets (733.29 ± 70.49 pg/mg vs 429.40 ± 73.17 pg/mg and 144.75 ± 14.65 pg/mg vs 40.15 ± 6.19 pg/mg respectively ($p<0.0001$)). Similar results were found for osteopontin, MMP-9, TIMP-1 and VEGF. In contrast, TFPI antigen was found to be much lower in these calcified regions (722.54 ± 153.92 pg/mg vs 2459.28 ± 285.36 pg/mg ($p<0.0001$)).

Conclusion — These results demonstrate that aortic valve lesions display several characteristics of atherosclerosis, including TF expression. In addition, we showed that TF is colocalized with calcification and lipid deposition. Further studies are now set up to evaluate the role of TF in aortic valve disease and its association with other components of the atherosclerotic process.

A009

IMPORTANCE OF TWEAK-CD163 SYSTEM IN PERIPHERAL ARTERY DISEASE

J.-A. MORENO [1], D. SMADJA [2], J.-L. MARTIN-VENTURA [3], J. EGIDO [3], L.-M. BLANCO-COLIO [3], J.-B. MICHEL [1], O. MEILHAC [1]
[1] *Inserm U698, Université Paris 7, CHU Xavier-Bichat, Paris, France*
[2] *Université Paris Descartes, 2AP-HP, Hôpital Européen Georges Pompidou, Inserm 765, Faculté de Pharmacie, Paris, France*
[3] *Vascular Research Lab. Fundación Jiménez Díaz. Autonoma University, Madrid, Spain*

Introduction — CD163 is a macrophage receptor of haptoglogin/haemoglobin complexes responsible for clearance of hemogloin. It has been recently suggested to be a potential scavenger receptor for TWEAK (Tumor necrosis factor-like weak inducer of apoptosis). TWEAK levels were reported to be decreased in carotid atherosclerosis. Our hypothesis is that decreased circulating TWEAK could be paralleled by an increased presence of CD163-expressing macrophage in atherosclerotic plaques. Since peripheral artery disease (PAD) is an important manifestation of systemic atherosclerosis, we have assessed the levels of circulating TWEAK-CD163 in PAD.

Methods and Results — Patients with PAD (n=184) had lower TWEAK (169.2±8.3 vs 211.9±15.4 pg/mL; $p<0.05$) and higher sCD163 (408.1±14.5 vs 317.4±8.4 ng/mL; $p<0.05$) plasma concentration than age-matched controls (n=330). After stratification according to the severity of disease, we observed that TWEAK/sCD163 ratio was significantly decreased in those patients with higher degree of disease (0.39±0.06 vs 0.66±0.08, $p<0.05$) relative to the other groups. Analysis of conditioned medium obtained from cultured human atherosclerotic femoral plaque samples (n=38) and healthy aortas (n=14) revealed that higher amount of sCD163 was released by the atherosclerotic tissue, whereas TWEAK presented the opposite trend.

Conclusions — Our results suggest that CD163/TWEAK plasma ratio could be a potential biomarker of clinical peripheral artery disease. We can hypothesized that decreased levels of circulating TWEAK observed in atherosclerosis may be the result of a trapping by plaque macrophages through their CD163.

A010

INTERFERENCE WITH TOLL-LIKE RECEPTOR 4 PATHWAY MEDIATES THE ANTI-INFLAMMATORY EFFECTS OF ADENOSINE

B. HAAS [1], F. LEONARD [1], I. ERNENS [1], M. VAUSORT [1], M. ROLLAND-TURNER [1], T. CHAN [2], A.-M. FELDMAN [2], Y. DEVAUX [1], D.-R. WAGNER [3]
[1] *Centre de Recherche Public — Santé, Luxembourg, Luxembourg*
[2] *Thomas Jefferson University, Philadelphia, USA*
[3] *Centre Hospitalier, Luxembourg, Luxembourg*

Purpose — Adenosine, acting through four types of receptors (A1, A2a, A2b, A3), is anti-inflammatory and cardioprotective. Since Toll-Like Receptor 4 (TLR4), a receptor involved in innate immunity, has recently been shown to mediate adverse left ventricular remodeling after myocardial infarction (MI), we sought to determine whether adenosine acts on the TLR4 pathway.

Methods — Primary human macrophages obtained after in vitro differentiation of blood monocytes isolated from healthy volunteers and patients with acute MI were treated with adenosine (10 μm), adenosine analogs, and/or lipopolysaccharide (LPS, 100 ng/mL). Transgenic mice bearing a cardiac-specific and externally-regulatable overexpression of A1 or A2a receptors were used to determine the receptor involved. Flow cytometry, immunoblotting, quantitative PCR and ELISA were used to

measure expression levels of TLR4, Tumor Necrosis Factor-α (TNF-α), TNF receptor-associated factor -6 (TRAF-6), and β-arrestins.

Results — Consistently with others, we observed that the TLR4 pathway is activated following MI. In LPS-treated macrophages, adenosine decreased cell surface expression of TLR4, resulting in a decrease of TNF-α production. This anti-inflammatory effect was also observed when macrophages were challenged with endogenous TLR4 ligands such as hyaluronic acid or heparan sulfate. Inhibition of TNF-α production by adenosine was stronger in macrophages isolated from MI patients compared with healthy volunteers. The effect was replicated by agonists of A2a receptors and blocked by antagonists of A2a receptors. Over-expression of the A1 and A2a receptors in mice both resulted in a decrease of TLR4 and TRAF-6 expression in the heart. Co-immunoprecipitation studies indicated that adenosine enhances the binding of β-arrestins to TRAF-6, thus interrupting TLR4 signaling.

Conclusion — Adenosine down-regulates inflammation through interference with the TLR4 pathway. This mechanism involves enhanced binding of β-arrestins to TRAF-6 in the presence of adenosine. This result may have important implications as it explains some anti-inflammatory and cardioprotective properties of adenosine.

A011

MATRIX METALLOPROTEINASE-12 GENE REGULATION BY PPAR ALPHA AGONIST IN HUMAN MONOCYTE-DERIVED MACROPHAGES

I. JGUIRIM-SOUISSI [1,2], L. BILLIET [2], C. CUAZ-PEROLIN [2], M.-N. SLIMANE [1], M. ROUIS [2]

[1] *Research laboratory on atherosclerotic biological and genetic factors, Faculty of Medicine., Monastir, Tunisia*

[2] *UMR 7079 Physiologie et Physiopathologie, Université Pierre et Marie Curie-Paris 6, CNRS, Paris cedex 5, France*

MMP-12, a macrophage-specific matrix metalloproteinase with large substrate specificity, has been reported to be highly expressed in mice, rabbits and human atherosclerotic lesions. Increased MMP-12 from inflammatory macrophages is associated with several degenerative diseases such as atherosclerosis. Our results showed that IL-1b, a proinflammatory cytokine found in atherosclerotic plaques, increased both mRNA and protein levels of MMP-12 in human monocyte-derived macrophages (HMDM). Since peroxisome proliferator-activated receptors (PPAR) such as PPARa and PPARg are expressed in macrophages and because PPARs activation exert an anti-inflammatory effect on vascular cells; we have investigated the effect of PPARa and g isoforms on MMP-12 regulation in HMDM. Our results showed that MMP-12 expression (mRNA and protein) was down regulated in IL-1b-treated macrophages only in the presence of a specific PPARa agonist GW647 in a dose-dependent manner. In contrast, this inhibitory effect was abolished in IL-1b-stimulated peritoneal macrophages isolated from PPARa-/- mice and treated with the GW647 PPARa agonist. Moreover, reporter gene transfection experiments using different MMP-12 promoter constructs showed a reduction of the promoter activities by ~50% in IL-1b-stimulated PPARa pretreated cells. However, MMP-12 promoter analysis did not reveal the presence of a PPRE response element. The IL-1b effect is known to be mediated through the AP-1 binding site. Mutation of the AP-1 site, which is located at -81 in the MMP-12 promoter region relative to the transcription start site, followed by transfection analysis and gel shift experiments revealed that the inhibitory effect was the consequence of the protein-protein interaction between GW 647-activated PPARa and c-Fos or c-Jun transcription factors, leading to inhibition of their binding to the AP-1 motif. These studies suggest that PPARa agonists may be used as a therapeutically, not only for lipid disorders, but also to prevent inflammation and atheromatous plaque rupture, where their ability to inhibit MMP-12 expression in HMDM may be beneficial.

A012

MESURE DE LA LONGUEUR DES TÉLOMÈRES SUR DIFFÉRENTS TISSUS ARTÉRIELS PROVENANT DE PATIENTS ATTEINTS D'ATHÉROSCLÉROSE

R. NZIETCHUENG [1], M. ELFARRA [2], C. LABAT [1], J. NLOGA [2], J.-P. CARTEAUX [2], P. MAUREIRA [2], G. POITEVIN [1], P. LACOLLEY [1], J.-P. VILLEMOT [2], A. BENETOS [1]

[1] *Inserm U961, Faculté de médecine, Nancy, France*

[2] *Service de Chirurgie Cardiovasculaire, CHU, Nancy, France*

Le raccourcissement des télomères est un déterminant majeur du vieillissement cellulaire. Des études in vitro montrent qu'un stress oxydant accru serait responsable d'une attrition plus marquée des télomères. Par ailleurs, des études cliniques portant sur l'association athérothrombose — longueur des télomères ont montré que les sujets athéromateux avaient des télomères leucocytaires plus courts. Le but de cette étude est d'étudier la dynamique des télomères sur des tissus atteints ou non par l'athérosclérose. Différents paramètres artériels (PAS et PAD) et métaboliques (diabète, dyslipidémie, BMI, tabagisme) ont été évalués au sein de 28 hommes et de 9 femmes (âge moyen : 70 ± 7,8 ans) présentant différentes pathologies cardiovasculaires. La longueur moyenne des télomères a été mesurée par Southernblotting sur des tissus artériels atteints de l'athérome, des tissus artériels non athérogènes, du tissus graisseux et du sang. Les tissus athérogènes de l'arbre aortique constitué par la valve aortique, l'aorte ascendante, l'aorte abdominale, l'artère iliaque et l'artère fémorale, présentent des télomères plus courts que ceux retrouvés dans les vaisseaux non athérogènes (artère mammaire et veine saphène). En présence de facteurs de risque cardiovasculaire (HTA, diabète type 2, tabagisme), seuls les tissus athérogènes présentent des TRF plus courts, les TRF des tissus non athérogènes étant essentiellement influencés par l'âge. Notre étude montre aussi qu'il existe, uniquement dans l'arbre aortique, une relation inverse entre la pression pulsée, indice de rigidité aortique, et la longueur des télomères. Les différences de TRF observées entre tissus sains et tissus athéromateux pourraient suggérer l'existence d'une régulation locale de la taille des télomères pouvant être due à la présence du processus inflammatoire caractéristique de l'athérosclérose, et d'un stress oxydant accru, ces deux processus étant fréquemment rencontrés chez les patients présentant des facteurs de risque cardiovasculaires tels que l'HTA, le diabète, et une augmentation de la rigidité artérielle.

A013

ETAT D'HYPERCOAGULABILITÉ AVEC ALTÉRATIONS ARTÉRIELLES CHEZ LE RAT ZUCKER

N. SLOBODA [1], R. SAID [1], H. LOUIS [1], K. HESS [1], C. LABAT [1], J.-P. MAX [1], S.N. THORNTON [1], P. CHALLANDE [2], P. LACOLLEY [1], V. REGNAULT [1]

[1] *Inserm U961 et Université Henri Poincaré, Vandoeuvre, France*

[2] *UPMC Université Paris 06, Paris, France*